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COMMENTARY

# An ion channel in the company of a transporter

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Ion channels and transporters share common 3D space on the surface of cells and within the plasma membrane, and recent evidence points toward physical and functional associations between them (Manville and Abbott, 2019). In the current issue of the *Journal of General Physiology*, Lamothe and Kurata explore the functional relationship between the Kv1.2 potassium channel, when the Kvβ1.2 β subunit is bound to the interior aspect of the channel, and Slc7a5, a component of the neutral amino acid transporter LAT1.

Voltage-gated potassium (Kv) channels are found in many, if not most, cells of the human body. They are tetrameric proteins that contain a pore through which potassium ions flow selectively (Long et al., 2005). There are 12 families of voltage-gated potassium channels, each grouped by shared sequences seen among the genes that code individual subunits (Alexander et al., 2015). These families share many functional and structural characteristics, but differences in their coding sequences impart distinctive features.

The mammalian Kv1 family of channels share a close genetic, structural, and functional relationship with the *Shaker* voltage-gated potassium channel that was discovered in the fruit fly (Papazian et al., 1987; Tempel et al., 1987; Baumann et al., 1988; Christie et al., 1989). In mammals, Kv1 channels are found in various types of cells, such as myocytes of the heart and neurons of the brain, and they are thought to underlie fast-activating potassium currents. The Kv1 channel is made up of individual subunits coded by one of seven specific genes, *KCNA1–KCNA7*, that can coassemble to form homo-tetrameric (Kv1.1–Kv1.7) or hetero-tetrameric structures (Christie et al., 1990; Isacoff et al., 1990; Ruppersberg et al., 1990). The Kv1 tetramer composition is likely cell-type specific, determined by select expression of particular KCNA genes (Sheng et al., 1993; Wang et al., 1993; Lorincz and Nusser, 2008). This divergent composition likely explains some of the differences observed in electrophysiologic behavior of Kv1 channels, seen among cell types.

Interestingly, even cells that do possess the same KCNA genes may exhibit differences in fast-activating potassium currents. A clue as to why the same Kv1 channel might behave differently in different cell types came from studies using dendrotoxin (DTX), a molecule that inhibits via binding to the external portion of a fast-activating potassium channel of unknown composition. Upon purification of the DTX-receptor from the brain, two proteins were isolated (Scott et al., 1990; Parcej et al., 1992). One of the proteins was the Kv1 channel subunit. The other was a protein that is now known to be the Kvβ subunit, which interacts with the N-terminal domain of the Kv1 subunit (subsequently referred to as the α subunit; Rettig et al., 1994; Scott et al., 1994). The β subunit can modify how potassium ions move through the channel pore formed by the α subunit tetramer, quickly reducing the flow of ions (called fast inactivation); it also regulates how efficiently the α subunit localizes to the plasma membrane (Shi et al., 1996; Accili et al., 1997b). However, not all Kv1 α subunits were found to be associated with a β subunit upon purification with the DTX-receptor (Parcej et al., 1992; Shamotienko et al., 1997). Thus, the variable presence of β subunits with the DTX-receptor provided early insight as to why differences in the electrophysiological signature of the Kv1 channel was observed in cell types that possess the same KCNA genes.

Since the discovery of the Kvβ subunits, other proteins have been proposed to associate with Kv1 channels and modify their behavior. For example, contactin-associated protein (Caspr) is thought to associate with the Kv1–Kvβ complex and, together, to localize in regions near the Node of Ranvier in the axons of nerves (Poliak et al., 1999). More recently, the σ-1 receptor, a transmembrane protein of the ER, has been proposed to form complexes with the Kv1.2 channel to modify their behavior and to influence electrophysiologic activity in the brain (Kourrich et al., 2013; Abraham et al., 2019). The evidence suggests that complexes of proteins may be the units that determine the location and function of Kv1 channels, and unique associations may be responsible for the observed variability (Trimmer, 2015). Kv1-containing complexes may also impact aspects of the channel life cycle (Deutsch, 2003), such as maturation and movement to the cell surface, as is the case for the Kvβ subunit (Shi et al., 1996), as well as removal from the cell surface and catabolism. For example, the association of Kv1.2 with the σ-1 receptor has been proposed to promote forward trafficking of the channel as well as to modulate its gating (Kourrich et al., 2013; Abraham et al., 2019), whereas the association of Kv1.2 with cortactin, an actin binding protein, is thought to increase endocytosis of the channel (Williams et al., 2007).

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An exciting development from the Kurata group at the University of Alberta has been the discovery of a novel Kv1 channel association between the transporter subunit Slc7a5 and the Kv1.2 channel (Baronas et al., 2018). The Slc7a5 protein is thought to heterodimerize with CD98hc (also known as Slc3a2) to form LAT1 (or the large amino acid transporter 1; Yan et al., 2019). LAT1 imports neutral amino acids, such as leucine, in exchange for intracellular amino acids, such as glutamine, in a sodium and pH-independent manner (Kanai et al., 1998; Mastroberardino et al., 1998). The initial discovery of the association between Scl7A5 and Kv1.2 was made by probing for channel-interacting proteins using mass spectroscopy and supported by several other high-resolution approaches. Imaging using antibodies specific to Slc7a5 and to Kv1.2 showed that the endogenous proteins are in close proximity in cortical neurons. Electrophysiology in mammalian cell lines that express Kv1.2, either with or without Slc7a5, showed that channel function was impacted by the transporter, as evidenced by a negative shift in the activation curve, promotion of slow inactivation, and slow closing. Notably, Baronas et al. (2018) also observed that modulation of Kv1.2 by the Kvβ1.3 subunit, which introduces rapid current inactivation, occurred in concert with a hyperpolarizing shift of the activation curve in the presence of the transporter. These provocative data suggest that the Kv1.2 channel is simultaneously associated with both Kvβ1.3 and Slc7a5, and they raise interesting questions about the nature of this interaction.

In the current issue of the *Journal of General Physiology*, Lamothe and Kurata (2020) thoughtfully use the structural and biophysical insights gained from studies of Kv1 channels and their interactions with Kvβ subunits to answer key questions about the association with Slc7a5.

Does Kvβ alter the functional interaction between Slc7a5 and Kv1.2? Slc7a5 was proposed by Baronas et al. (2018) to inhibit the Kv1.2 channel by stabilizing a nonconducting state. Inhibition was inferred from experiments showing that when the cells were held at a very negative voltage (−120 mV) and depolarized to a positive voltage (10 mV) for 50 ms every 2 s, the amplitude of the current becomes larger over time. This growth in current amplitude suggests that the channel was progressively disinhibited by the voltage protocol. In support of this interpretation, no increase in current was observed when the same protocol was used in the absence of Slc7a5 channel.

Lamothe and Kurata (2020) use a parallel approach to show that disinhibition of Kv1.2 currents by Slc7a5 also occurs with the full-length Kvβ1.2 (though to a lesser extent), whereas it was not affected by a β subunit that lacks a region of the N-terminus, the ball and chain region, that enters the pore and causes the quick reduction in current (fast inactivation; Gulbis et al., 2000). Then, when the channel was subsequently held at an even more negative voltage (−160 mV), disinhibition of the Kv1.2 current by Slc7a5 occurred to a *greater* extent in the presence of the full-length Kvβ1.2 subunit; again, no effect was observed with the N-terminally truncated Kvβ1.2 subunit. Thus, the Slc7a5-induced inhibition of the Kv1.2 channel was shifted to more negative voltages by the Kvβ1.2 N-terminus. This shift is reminiscent of the promotion of C-type inactivation by N-type inactivation in the *Shaker* channel, where either its own N-terminus or that of Kvβ1.2 inhibits the entry of potassium into the pore and a nonconducting conformation is thought to result more easily (López-Barneo et al., 1993; Baukrowitz and Yellen, 1995; Rasmusson et al., 1995; Morales et al., 1996).

Does Slc7a5 alter the interaction between Kvβ and Kv1.2? The Kvβ1.2 protein, upon binding to the Kv1.2 N-terminus, induces a pronounced fast reduction of delayed rectifier current (fast inactivation), in mouse LM cells. However, when all three proteins were coexpressed in these cells the rate and extent of Kvβ-induced inactivation were increased. Notably, the greater rate of inactivation induced by Slc7a5 occurred when this effect of Kvβ1.2 binding to the Kv1.2 channel was saturated. Thus, a mechanism other than varying the Kvβ-Kv1.2 stoichiometry, which may account for altered rates of inactivation (Accili et al., 1997b, 1997a; Xu et al., 1998; Jing et al., 1999; Schulte et al., 2006), is probably responsible for the greater rate of Kvβ-induced inactivation induced by Slc7a5.

Lamothe and Kurata (2020) also observed a greater delay in recovery from inactivation and greater slowing of current deactivation of the Kv1.2/Kvβ1.2 channel complex when in the presence of Slc7a5. These known effects of Kvβ1 subunits are thought to be due to the required exit of the N-terminal domain from the pore before the channel can deactivate and close (England et al., 1995; Morales et al., 1996; Accili et al., 1997b; De Biasi et al., 1997; Peters et al., 2009). To explain the altered electrical behavior of the channel-β subunit when in the company of the transporter, the authors propose a simple model: a more stable inactivated conformation of the pore from which the N-terminal domain of Kvβ1.2 unbinds more slowly. How Slc7a5 might stabilize a nonconducting state of the pore is not yet clear but one could imagine that it results from an interaction either direct or indirect that promotes a pore structure which reflects C-type inactivation.

The temporal and physical nature of the Kv1/Kvβ1.2/Slc7a5 association has yet to be fully understood. Lamothe and Kurata (2020) show that coexpression of the transporter reduces Kv1.2 protein expression, as well as current density. By contrast, Kvβ1.2 augments protein expression of Kv1.2 and limits the reduction in channel protein brought about by Slc7a5. Kvβ1.2 also increases current density as well as the levels of immature and mature bands, which is consistent with an increase in protein, surface expression, and number of channels as suggested previously (Shi et al., 1996; Accili et al., 1997b). Kvβ1.2 reverses the drop in current density and in the amounts of mature and immature bands brought when Slc7a5 is coexpressed. This could mean that there is a competition between Kvβ1.2 and Slc7a5 for binding to the Kv1.2 channel. Kvβ is thought to associate strongly with the proximal N-terminus of the Kv1.2 channel early in protein biogenesis in the ER, after which the α and β proteins move to the plasma membrane together (Shi et al., 1996; Nagaya and Papazian, 1997). Thus, the interaction between transporter and channel may occur at the same point as the β subunit and channel during biosynthesis and export, before their arrival at the plasma membrane (Deutsch, 2002).

The findings of Lamothe and Kurata (2020) show that effects of Kvβ1.2 and Slc7 a5 are not in direct opposition. Kvβ1.2, unlike Slc7a5, appears to preferentially increase the mature band with or without coexpression of Slc7a5. Co-immunoprecipitation

(co-IP) of Kv1.2 and Kvβ1.2 show that their association may be stabilized when Slc7a5 is coexpressed. These two findings argue against a direct competition between Kvβ1.2 and Slc7a5 and leave open the possibility that their physical and temporal association with the channel is not the same. For example, the transporter may associate with the channel complex initially and indirectly once they both arrive at the cell surface, which could then influence events both upstream and downstream. However, the plasma membrane is thought to be a crowded space (Ryan et al., 1988) and ion channels and transporters may not, necessarily, be overrepresented there. Therefore, finding each other and maintaining a stable interaction may require direct or indirect associations before arriving at the cell surface either during or after translation or perhaps even earlier by association of transcripts as has been proposed for hERG voltage-gated potassium channels and voltage-gated sodium channels expressed in myocytes of the heart (Eichel et al., 2019). Further study will be required to understand when and where the association between channel and transporter takes place and the impact of the interaction on the full life cycle of the α–β channel complex.

The stabilization of the Kv1.2 and Kvβ1.2 interaction by Slc7a5, as shown by co-IP in Lamothe and Kurata (2020), is consistent with an altered physical state or structure of the α–β complex and the resulting changes in its electrical function. Based on these findings, a physical model could be envisaged whereby the structure of the regions of the α–β interaction, namely the N-terminal domains of the channel and the C-terminal domains of the β subunit, are impacted by Slc7a5. The changes induced in these regions by Slc7a5 could propagate throughout the channel complex and ultimately reach domains that influence the unbinding N-terminal domain of Kvβ1.2 from the Kv1.2 pore and slow this process. Here, too, more work will be necessary to better understand the physical basis of how the α–β complex is associated with Slc7a5 and, importantly, how their association with the transporter changes their interaction with each other.

Insight into the physical and function interactions between Kv1.2 and Slc7a5, together with evidence of their co-localization in hippocampal neurons, opens a window into how this novel association might influence electrophysiologic behaviour in vivo. Such an association could help to explain the variability of currents that arise from the Kv1.2 channel, and of resulting electrical activity, between individual neurons (Baronas et al., 2015). Also, reciprocal regulation of amino acid transport by the association with Kv1.2 may be of relevance in vivo given that, to form LAT1, Slc7a5 associates with CD98hc (Slc3a2). This single-pass transmembrane protein does not appear to partner with Kv1.2 on its own, but it reduces the impact of Slc7a5 on the Kv1.2 channel, perhaps by competing with the channel for their common partner (Baronas et al., 2018). Finally, this association will likely yield significant insight into the precise roles of these proteins in the body and how they cause disease when they do not work properly, which remains difficult to ascertain (Murphy et al., 2004; Brew et al., 2007; Syrbe et al., 2015). Indeed, gain-of-function missense mutations in Kv1.2, which are linked to epilepsy, are more sensitive to Slc7a5 while missense mutations in Slc7a5 (Baronas et al., 2018), which are associated with autism spectrum disorder and motor delay (Tărlungeanu et al., 2016), diminish its effects on the Kv1.2 channel.

The discovery of the interaction of the Kv1.2/Kvβ1.2 channel complex with Slc7a5 is among a growing number of channel-transporter associations and may represent a common mode of signaling in cells (Manville and Abbott, 2019). These associations may help to explain how variable electrophysiologic phenotypes can be bestowed upon different cells expressing the same ion channel gene. They will also help to understand the role of transporters and how that may vary between cells that do and do not express their ion channel partner. Sophisticated analyses of how interactions between ion channels and transporters influence the function of individual components, as realized here by Lamothe and Kurata (2020), are necessary to understand their significance.

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